EPA REGION 1

STANDARD OPERATING PROCEDURE FOR THE ANALYSIS OF POLINUCLEAR HYDROCARBONS (PAH) IN AQUEOUS SAMPLES USING SIM-GC/MS ANALYSIS

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1.0 Summary:

A measured volume of the sample at neutral pH is serially extracted with methylene chloride using separatory funnel. The extract is dried and concentrated. For very dirty samples silica gel cleanup must be done. The extract is analyzed by GC/MS-SIM

2.0 Scope and Application:

This method is for determination of polynuclear hydrocarbons from water and applicable for the following compounds.

Reporting Limits

Naphthalene	Phenanthrene	Benzo(b)fluoranthene
2-Methylnaphthalene *	Anthracene	Benzo(k)fluoranthene
1-Methylnaphthalene *	Fluoranthene	Benzo(a)pyrene
Acenaphthylene	Pyrene	Dibenzo(a,h) anthracene
Acenaphthene	Benzo(a)anthracene	Benzo(g,h,i)perylene
Fluorene	Chrysene	Indene(1,2,3-cd)pyrene

*- optional PAH

Reporting limit for 1.0L of sample = $\frac{\text{FV (1) mL *RL (0.05ug/ml)}}{\text{1 liter}}$ = 0.05ug/L

RL = low standard concentration, ug/mL

3.0 Definitions:

3.1 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component. The compounds are napthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d10, and perylene-d12.

Final concentration in the sample or standard - 2 ug/mL.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of a SA is to monitor method performance with each sample. The compounds are2- fluorobiphenyl , and terphenyl-d14 at 4ug per sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC)/ Tuning Solution -- A solution of one or more method analytes and or other test substances used to evaluate the performance of the instrument system with respect to a defined set of method criteria. The compounds are pentachlorophenol, DFTPP, benzidine and DDT.

3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (MS) -- An aliquot of an

environmental sample to which known quantities of the method analytes are added in the laboratory. The MS is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations, 4 ug per sample for all target analytes are used.

3.10 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE(MSD) -- Second aliquots of the same sample taken in the laboratory to which known quantities of the method analytes are added in the laboratory and analyzed separately with identical procedures. Analyses of MS and MSD indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.11 STOCK STANDARD SOLUTION -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.12 WORKING STANDARD SOLUTION -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.13 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.14 SECONDARY STANDARD - A standard from another vender that is used to check the primary standard used for quantitation.

4.0 Safety:

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these reagents should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should be made available to all personnel involved in these analyses. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing, and observe proper mixing when working with these reagents.

4.2 Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions

of these compounds should be handled with suitable protection to skin, eyes, etc.

5.0 Personnel qualifications:

5.1 The analyst should have at least four year degree in physical science.

5.2 The analyst must have a satisfactory IDC/MDL in place before analyzing samples.

5.3 All personnel shall be responsible for complying with all quality assurance/quality control requirements that pertain to their organizational/technical function.

6.0 Interferences:

Contaminants in solvents, reagents, glassware, and other sample processing hardware may cause method interferences such as discrete artifacts and/or elevated baselines in the extracted ion current profiles (EICPs). All of these materials routinely must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Matrix interferences may be caused by contaminants that are co extracted from the sample. The extent of matrix interferences will vary considerably from source to source.

7.0 Equipment and Supplies:

7.1 Beakers – various volumes

7.2 Syringes - 2 µL, 10 µL, 0.2 mL, 0.5 mL and 10 mL with Luerlok fitting.

7.3 Pasteur Pipets - 1 mL glass, disposable.

7.4 Vial and Caps - amber glass, 2 mL capacity with Teflon-lined screw cap, 2 mL capacity for GC auto sampler.

7.5 Balances - analytical, capable of accurately weighing ± 0.0001 g and one capable of weighing 100 g to ± 0.01 g. The balances must be calibrated with class S weights or known reference weights. The balances must also be annually checked by a certified technician.

7.6 pH Paper

7.7 Concentrator Tubes -15 or 10 ml graduated.

7.8 Pyrex Glass Wool – rinsed with methylene chloride

7.9 Silicon Carbide Boiling Chips – approximately10/40 mesh. Heat to 400 C for 30 minutes.

7.10 Test Tube Rack.

7.11 Volumetric flask 5ml,10 ml.

7.12 Reagent Water - defined as water in which an interfering compound is not observed at or above the CRQL for each analyte of interest.

7.13 Acetone, methylene chloride, cyclohexane ,pentene - pesticide residue analysis grade or equivalent.

7.14 Sodium sulfate – powdered or granular anhydrous reagent grade, heated at 400 C for 4 hours in shallow tray cooled in desiccator and stored in a glass bottle.

7.15 Silica Gel – 100/200 mesh(Davison Chemical grade 923 or equivalent) Before use activate for at least 16 hour at 130C in shallow glass tray , loosely covered with aluminum foil.

7.16 Turbo Vap – nitrogen blow down system.

7.17 Organomation KD Concentrator.

7.18 Concentration flask, Zymark, 50 mL.

7.19 Snyder column- Two-Three ball macro (Kontes, k-503000-0121).

7.20 Evaporation flask –300 ml (Kontes k-570001-500 or equivalent).

8.0 Reagents and Standards:

8.1 Calibration Solutions (Cals):

Primary Stocks:

Supelco , TCL Polynuclear Aromatic Hydrocarbons Mix ,2000 ug/ml in methylene chloride.

8.2 Working standard (WS) 20 ug/ml

Combine 0.05 ml of Supelco calibration mix and 0.1 ml of B/N surrogate mix in 5 ml volumetric . Bring to volume with methylene chloride.

Calibration curve standards are all made with final volume 1.0 ml and all dilution are made with methylene chloride:

0.05 ug/ml std - 2.5 ul WS + 10 ml IS (section 8.6)

0.20 ug/ml std - 10 ul WS + 10 ml IS

1.0 ug/ml std - 50 ul WS + 10 ml IS
4.0 ug/ml std - 200 ul WS + 10 ml IS
8.0 ug/ml std - 400 ul WS + 10 ml IS
20.0 ug/ml std - 1 ml WS + 10 ml IS

8.3 Secondary standard and LFB/LFM stock
The ULTRA or Supelco standard but from a different lot are used to make the secondary standard. 0.1 ml of Ultra PAH mixture solution to 50 ml with acetone. 1 ml of the solution spiked to LFB and LFM in the batch.

8.4 Surrogate standard spiking solution: 0.2 ml of Restek B/N surrogate solution to 50 ml with acetone. 1 ml of the solution spiked to each sample in the batch.

8.5 Instrument performance check standard solution. GC/MS Tunning standard for EPA method 624/625, Acustandard.
Prepare a solution of decafluorotriphenylphosphine (DFTPP), such that a 1-2 μL injection will contain 50 ng of DFTPP.

8.6 Internal Standard Solution. Semivolatile Internal Standard Mix, Supelco , 2000 ug/mL, napthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d10, and perylene-d12. Working solution - add 0.1 ml of 2000 ug/mL Supelco IS in 1 mL volumetric flask and dulute with methylene chloride. Spike 10 uL per 1 mL of sample and standard for a 2.0 ug/mL concentration

8.7 Fresh working standards must be prepared once every twelve months, or sooner if standards have degraded or concentrated. Stock standards must be checked for signs of degradation or concentration just prior to preparing secondary dilution and working standards from them. Protect all standards from light. Samples, sample extracts and standards must be stored separately.

Holding times for working standards:
Surrogate mix - 3 months
Spike mix - 6 months
IS - by expiration date.

9.0 Initial and Continuing Calibration Solutions.

9.1 Prepare calibration standards at a minimum of five concentration levels (0.05, 0.2, 1.0, 4.0, 8.0 , and 20.00 ug/mL). Each calibration standard must contain each compound of interest and each surrogate. The acceptance criteria is $\pm$ 20.0% RSD for the average initial calibration. Quantitation is done by using the average response factors

or linear regression.

9.2 Middle range secondary standard is used as a continuing calibration standard and must be run every 12 hours. Acceptance criteria are 20% D from the initial average response factors.

10.0 Sample preparation:

10.1 Transfer the entire contents of the sample to separatory funnel. Add 1.0mL of the 4 ug/mL B/N surrogate mix to all samples in the batch, including QC samples. Add 1.0 ml of 4.0 ug/ml (WS) matrix spike solution to the LFB, /LFB duplicate, MS/MS duplicate. Use first 60 ml portion of methylene chloride to rinse bottle and transfer this rinse solvent to the separatory funnel.
Seal and shake the separatory funnel vigorously for 2 minutes with periodic venting to release excess pressure. ***Methylene chloride creates excessive pressure very rapidly; therefore initial venting should be done immediately after funnel has been sealed and shaken once.***

10.2 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must use various techniques to complete the separation. The optimum technique depends upon the sample and may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the solvent extract in an Erlenmeyer flask with glass funnel filled with baked sodium sulfate.

10.3 Repeat the extraction two more times using fresh portions of solvent. Combine the three solvent extracts.

10.4 Dry the extract during collecting the extract in Erlenmeyer flask with baked sodium sulfate.

10.5 Perform concentration using Kuderna-Danish technique and nitrogen blow down.

K-D technique:

10.5.1 Assemble a Kuderna-Danish (K-D) concentrator by adding one or two clean boiling chips and attaching a 10-ml concentrator tube with 1 ml point end to a 250 or 500-ml evaporation flask.

10.5.2 Attach a three-ball KD column and the solvent vapor recovery glassware (condenser and collection device) to the Snyder column of the K-D apparatus.

10.5.3 Transfer extract from Erlenmeyer flask to evaporation flask via three-ball KD column Place the K-D apparatus on a hot water bath (15 - 20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 10 - 20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches below 10 ml, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

10.5.4 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 ml of methylene chloride or exchange solvent. The sample volume can be set around 10 ml. Transfer extract to 50 mL concentration flask with 1 ml point end.

10.5.5 Using Nitrogen Evaporator concentrate the sample to 1 ml at 45C with 10 psi of nitrogen using volume sensor.

10.6 If the extract does not need silica gel clean up transfer the extracts to a 1 ml Teflon-sealed vial , spike with 10ul IS (200ug/Mll) per 1 mL of sample . Label and store ad 4C° . Extract must be analyzed within 40 days.

10.7 If the sample extract is very dirty, a silica gel clean up must be done. Before the silica gel cleanup technique can be utilized, the extract solvent must be exchange to cyclohexane.

The exchange is performed by adding 4-8 mL of cyclohexane to the sample extract (1-2-mL)and reduction to the final volume to 2.0 ml using nitrogen evaporation.

Caution : When the volume of the solvent reduced below 1.0 mL , naphthalene may be lost. If the extract goes to dryness, the extraction must be repeated.

10.8 Silica Gel Clean up.

10.8.1 Prepare a slurry of 10 g of activated silica gel in methylene chloride and place this into a 10 mm ID chromatographic column. Tap the column to settle the silica gel and elute the methylene chloride. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the silica gel. Elute the methylene chloride to the top of the silica gel. Do not expose the column to air.

10.8.2 Pre-elute the column with 40 ml of pentane. The elution rate for all elutions should be about 2 ml/min. Just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL cyclohexane sample extract onto the column using an additional 2 mL cyclohexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 25 mL of pentane and continue the elution of the column. Discard this pentane eluate.

10.8.3 Next, elute the column with 25 mL of 40 % methylene chloride/pentane (2:3 v/v) and collect this portion into a 25 mL concentration tube.

10.8.4 Concentrate the samples as outlined above by nitrogen evaporation and proceed to step 10.5

11.0 Quality Control:

This SOP is used in conjunction with the EIA-GCMS6 SOP

11.1 The GC/MS system must be tuned to meet the DFTPP criteria.

11.2 There must be an initial calibration of the GC/MS system.

11.3 The GC/MS system must meet the calibration verification acceptance criteria every 12 hours.

11.4 The RRT of the sample component must fall within the RRT window of the standard component.

11.5 Quality Control Samples - At a minimum, this includes the analysis of a method blank, matrix spike, a duplicate or a matrix duplicate.

11.6 Surrogate recoveries - The addition of surrogates to each field sample and QC sample must be done. The laboratory must evaluate surrogate recovery data from

individual samples versus the surrogate control limits developed by the laboratory.

11.7 The experience of the analyst performing GC/MS analyses is invaluable to the success of the methods. Each day that analysis is performed, the calibration verification standard should be evaluated to determine if the chromatographic system is operating properly. Careful examination of the standard chromatogram can indicate whether the column is still performing acceptably, the injector is leaking, the injector septum needs replacing, etc. If any changes are made to the system (e.g., the column changed, a septum is changed), whether recalibration of the system must take place.

12.0 Pollution Prevention and Waste Management

NERL encourages all chemist and biologists to investigate micro analytical techniques, innovative technologies, and chemical substitution in laboratory processes to reduce waste and prevent pollution. As analytical SOPs are reviewed, on an annual basis, the responsible chemist or biologist will incorporate waste minimization practices where practicable and where these practices have been demonstrated to return data of equivalent quality.

Chemists and biologists must refer to the Waste Management Program SOP for proper disposal of laboratory waste. Personnel should contact the Environmental, Safety and Health Department if changes in the analytical SOP will generate new waste streams. Questions regarding the proper disposal of laboratory waste and purchase of new reagents should be directed to the Environmental, Safety and Health Department in advance of actually initiating a change in the analytical method.

13.0 Archiving Data:

Copy the HP raw files (*.d), and the calibration files to a storage device at least annually.

14.0 Laboratory Documentation:

14.1 The certificates of analysis received with the standard are kept in the standard log book and kept in the laboratory room 198
14.2 Standards and reagents preparation information is documented in Standard Preparation log .The date of preparation, vendor, lot number, solvent, expiration date, date of receipt are documented. The standard preparation log is kept in the laboratory room 198.
14.3 The preparation of the sample is documented on the preparation summary sheets. The summary sheets documents the date, project number, analyst initials, sample number, sample weight, volumes of the reagents reagent used in the sample preparation, expiration date of reagents, detailed description on how the each sample in the batch was extracted and concentrated and final sample preparation and calibration .
Any deviation from normal procedure should be documented. Each page need to have

initial, the date, project number and survey name. All forms are placed in the project folder when completed.

15.0 References:

US EPA RCRA, Method 8270C, Semi-Volatile Organic Compounds by Gas Chromatography/ Mass Spectrometry (CG/MS):Capillary Column Technique

US EPA RCRA, Method 3510C, Separatory Funnel Liquid-Liquid Extraction

US EPA RCRA, Methos 3630C, Silica Gel Cleanup

EIASOP-BNAGCMS Revision 6 GC/MS Semivolatile Organics 1.27.2006

Table 1

Method Control Parameters

DFTPP tuning criteria

GC/MS PERFORMANCE STANDARD

Decafluorotriphenylphosphine (DFTPP)

m/Ion	Abundance Criteria
51	30-60% of mass 198
68	<2% of mass 69
69	0.01 - 100
70	<2% of mass 69
127	40-60% of mass 198
197	<1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	>0.75 % of mass 198
441	0-100% of mass 443
442	40-100% of mass 198
443	17-23% of mass 442

GC Method File: PAHSIM

Back Inlet(split/splitless)

Mode – split

Constant flow

Inlet temp: 250C°

Split ratio 5:1

MS source temp 260C°

MS quad temp 150C°

Transfer line temp 300C°

Injection volume -2 ul

GC temperature

Initial temp 40		Initial time	2.0 min
Rate 1	20C/min		
Temp 2	290C°		
Rate 2	2C/min		
Temp 3	303 C°		
Rate 3	6.0C/min		
Temp:4	330C°	Final time	1.0 min

MS Acquisition parameters

Acquisition mode SIM

Solvent Delay: 6 min

Group 1	Group 2	Group 3	Group 4	Group 5
Start/Stop, (6/9.9)	Start/Stop, (9.9/11.8)	Start/Stop (11.8/14.8)	Start/Stop (14.8/17.10)	Start/Stop (17.10/END)
M/Z Dwell	M/Z Dwell	M/Z Dwell	M/Z Dwell	M/Z Dwell
68 -50	152-50	80-50	120-50	125-50
127-50	153-50	94-50	125-50	138-50
128-50	154-50	101-50	266-50	139-50
129-50	160-50	176-50	228-50	227-50
136-50	162-50	178-50	229-50	252-50
137-50	164-50	179-50	236-50	253-50
172-50	165-50	188-50	240-50	260-50
	166-50	202-50	252-50	264-50
	167-50	203-50	253-50	265-50
		244-50		276-50
				277-50
				278-50
				279-50

Acceptance Criteria for PAHs by GC/MS

Audit	Frequency	Limits	Corrective Action
Laboratory Blank	One blank per extraction batch	Less than the RL of any single target compound	Samples associated with a contaminated blank must be re extracted and reanalyzed if enough sample is available and re extraction can be done within holding times. Otherwise, the client will be notified and if no re sampling is done target compounds associated with contamination are flagged in the report.
Field Blank or Equipment Blank	Depending on site	Less than the RL of any single target compound	Client must be notified of contamination. If re sampling is not done, target compounds associated with contamination are flagged in the report.
Matrix Spike	1 MS per batch of samples	±60% until control charts developed	Explain in project narrative. Action taken at data validation level.

Audit	Frequency	Limits	Corrective Action
PE Sample	Depending on site		Action taken at data validation level
DFTPP tune	Prior to analysis of all QC samples and blanks / every 12 hours	SOP criteria	Reanalyze all samples with non compliant DFTPP tune
Initial Calibration	All standards are analyzed prior to running the field and QC samples	±20% to use average RF;R Of at least .99 for linear calibration	Check septum, linear, column, rerun sequence. If there is still a problem, new standards must be prepared.
Continuous Calibration	Every 12 hours and at the end of each sequence	20%RSD for average RF; 30%D of true value for linear cal.	Check septum, liner, column, rerun sequence. If there is still a problem, rerun IC.
Internal Standard	All field and QC samples	Areas: ± 50% of the last CC Retention Times: ± 30 s from last CC	Rerun sample, if still a problem estimate values for that sample.
Surrogate Recoveries	All field and QC samples	± 60% until control charts developed	Rerun sample, if still a problem, re extract if within holding time. Otherwise, explain in project narrative. If all recoveries

Audit	Frequency	Limits	Corrective Action
			below 10%, notify client.
MDL*	when there is instrument change	Below RL for all target compounds	RL raised to value above MDL that is inclusive of the calibration range.
Retention Times of target analytes	Cal std and QC samples	Compounds found	Use graphic edit to check and manually integrate the compound, all of the samples must be checked for similar interference. If the interference cannot be resolved, the compounds data is estimated.
IDC*	When there is an extraction or instrument change	Extract and analyze 4 LFB Compare accuracy and precision results to lab generated control charts.	Investigate problem and correct. If limits are still not meet re-extract and re-analyze.